IFAPA

EUBerry Final Meeting Skierniewice

Task 1.3. Functional analysis of strawberry genes

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EUBerry

Task 1.3 Validation of the role of key genes in strawberry traits

Sub-task 1.3.1 Validation of nutritional quality-related genes
Sub-task 1.3.2 Validation of flowering-related genes

The third task will be the identification of strawberry genes controlling key traits.

This will be done by using appropriate genomics approaches, with the outcomes:

(i) Analysis of a segregating population of cultivated strawberry using expression profiles of candidate genes derived from microarray data (P5).

(ii) Characterisation of transgenic cultivated strawberry containing candidate genes.

(iii) Confirmation of candidate genes.

This task aims to confirm the function of some of the key genes involved in flowering and fruit quality traits in strawberry.

Task 1.3 Functional analysis of strawberry genes

- **Identification of genes differentially expressed in lines with low & high L-AA content**

  3 QTLs control aprox. 45% of the variation of L-AA content in pop. 232 x 1392

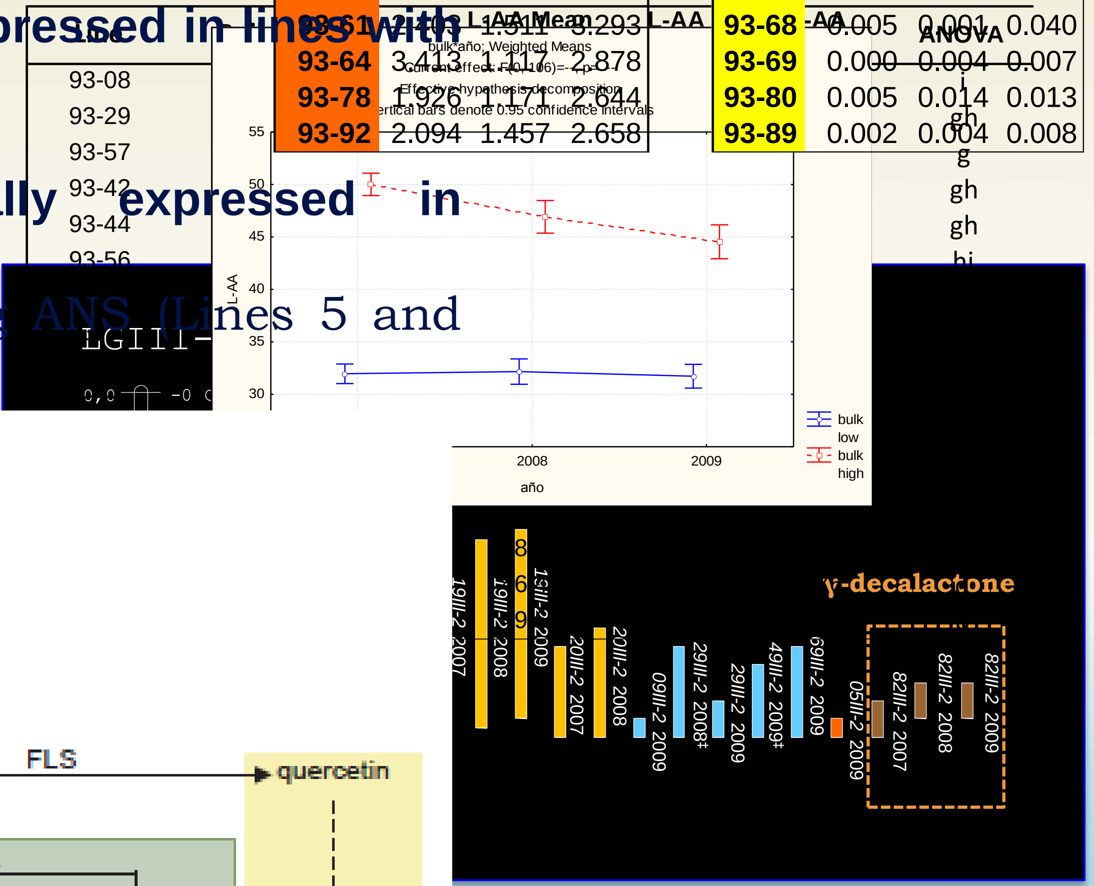
- **Identification of genes differentially expressed in lines with & w/o γ-decalactone**

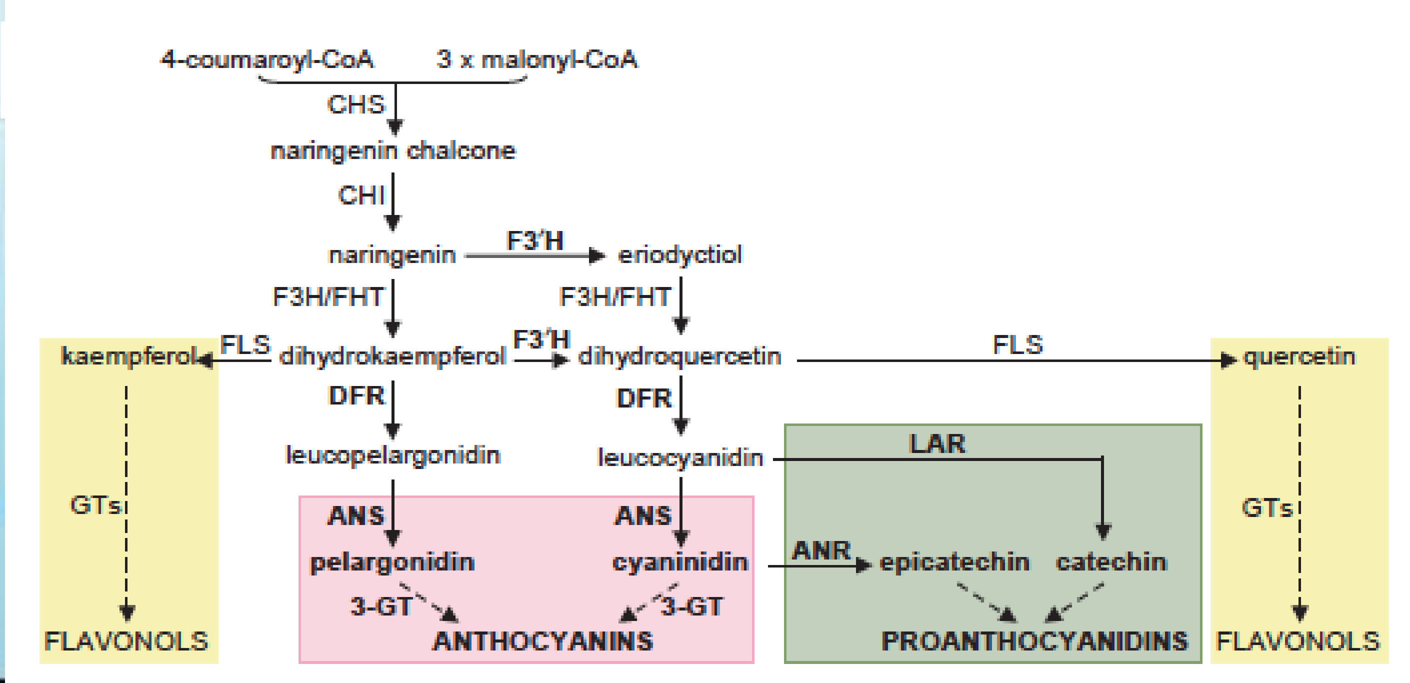
- **Identification of genes differentially expressed in transgenic lines overexpressing ANS**

  Transgenic lines of P1 overexpressing ANS (Lines 5 and 18) versus control 'Calypso' line.

with γ-decalactone			
line	2007	2008	2009
93-01	4.456	1.287	2.849
93-12	3.114	1.185	1.875
93-19	3.101	4.300	2.865
93-36	3.022	3.884	3.182
93-43	4.237	3.394	3.074
93-54	3.675	3.376	3.902
93-61	[illegible]	1.511	3.293
93-64	3.413	1.117	2.878
93-78	1.926	1.171	2.644
93-92	2.094	1.457	2.658

w/o γ-decalactone			
line	2007	2008	2009
93-03	0.000	0.019	0.014
93-07	0.002	0.001	0.005
93-14	0.010	0.007	0.019
93-17	0.001	0.003	0.002
93-18	0.001	0.008	0.006
93-49	0.009	0.007	0.010
93-68	0.005	0.001	0.040
93-69	0.000	0.004	0.007
93-80	0.005	0.014	0.013
93-89	0.002	0.004	0.008

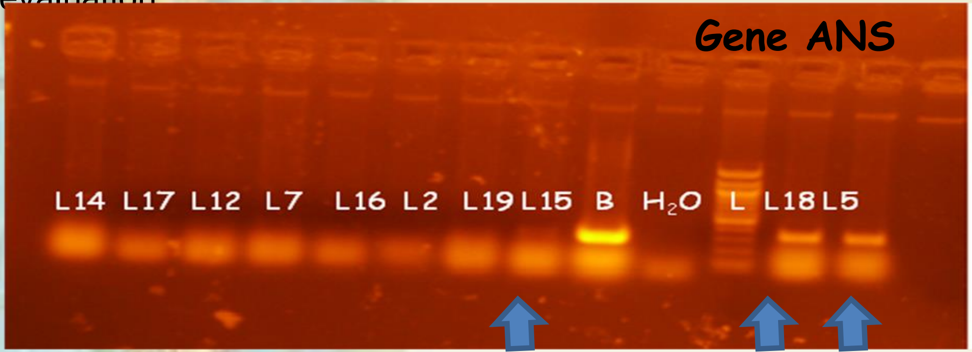




Task 1.3 Validation of the role of key genes in strawberry traits

Sub-task 1.3.1 Validation of nutritional quality-related genes

Transformation of varieties of Sveva and Calypso with 35S-driven ANS and DFR constructs mediated by *Agrobacterium tumefaciens* using strawberry leaves propagated in vitro. Strawberry transformed leaf are in selection phases on regeneration and selection substrate added with Kanamycin. Plants were propagated and grown in green house following the rules of GM restricted conditions evaluation.



PCR analysis of ANS gene has been done on propagated "Calypso" plants generated from transformation. Three lines, L15, L18 and L5 of gm plants resulted positive to ANS gene.

DFR plants has been analyzed by PCR with DFR gene and NPTII gene for Kanamicyn resistance. DFR plants resulted negative to PCR analysis of DFR gene, while resulted positive to NPTII gene.

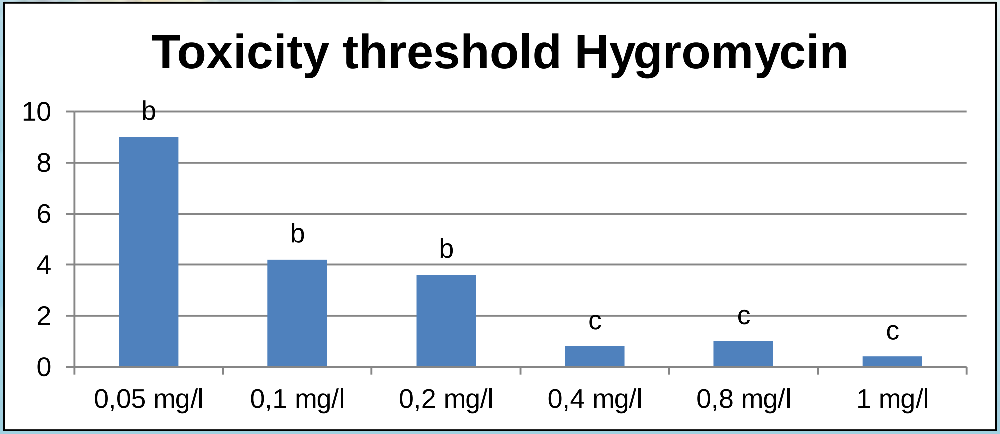
Investigation research are now mainly focused on ANS GM plants L5, L15 and L18 from which have been harvested fruits that has to be sent to P5 fro RNA extraction.

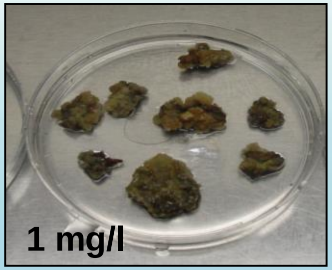
Sub-task 1.3.2 Validation of flowering-related genes

Transformation of varieties Sveva and Calypso have been started by P1 with constructs CaMV35S::FvKSN in pMDC32 (functional KSN) and CaMV35S::Fvksn in pMDC32 (non-functional ksn with the 2pb deletion in the first exon), from P6, mediated by *Agrobacterium tumefaciens,* were performed *using* strawberry leaves propagated in vitro. Ipotesis are: **CaMV35S ::FvKSN, homologue of *A.thaliana* TFL1 repressor of flowering delays the flowering date or suppresses flowering.**

Transformation of varieties Sveva and Calypso have been started by P1 with constructs harbouring homologues of *A.thaliana* activator of flowering FT1; FT2; FT3. **Ipotesis are a possibility to promote early flowering in SD Sveva.**

First step: *selective agent concentration, Hygromycin*





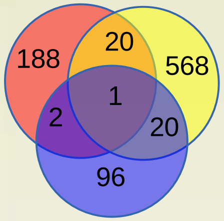
Leaf selection
conc. 0,5 – 1 mg/l

Plant selection
conc. ≤ 10 mg/l

RNA-Seq Calypso transgenic lines

ID	Total Reads	Clean Reads	Paired in sequencing		Properly paired	With itself and mate mapped	Singletons	With mate mapped to a different chr
			Reads	Pair 1				
		Aver.	Aver.	Pair 2	(nan%)		(nan%)	mapQ>=5
Calypso C1	37.64 M	36.48 M	50.01 M	25.21 M	33.44 M	40.44 M	9.57 M	0.25 M
		96.93%	68.54%	24.79 M	66.88%		19.13%	0.10 M
Calypso C2	38.98 M	37.83 M	52.54 M	26.49 M	34.25 M	42.09 M	10.46 M	0.25 M
		97.04%	69.44%	26.06 M	65.19%		19.90%	0.10 M
Calypso C3	38.09 M	36.99 M	50.74 M	25.59 M	33.74 M	40.93 M	9.81 M	0.24 M
		97.10%	68.59%	25.15 M	66.50%		19.33%	0.09 M
Calypso L5 1	33.12 M	32.11 M	43.61 M	21.99 M	29.49 M	35.57 M	8.04 M	0.22 M
		96.95%	67.91%	21.62 M	67.63%		18.43%	0.09 M
Calypso L5 2	42.71 M	41.52 M	56.51 M	28.52 M	38.66 M	46.06 M	10.45 M	0.27 M
		97.21%	68.05%	27.99 M	68.42%		18.50%	0.11 M
Calypso L5 3	37.45 M	36.32 M	50.32 M	25.38 M	34.52 M	40.98 M	9.34 M	0.25 M
		96.99%	69.28%	24.94 M	68.59%		18.56%	0.10 M
Calypso L18 1	37.73 M	36.77	50.42 M	25.42 M	37.45 M	41.71 M	8.72 M	0.21 M
		97.46%	68.57%	25.00 M	74.28%		17.28%	0.09 M
Calypso L18 2	34.14 M	33.34	45.47 M	22.87 M	31.80 M	37.02 M	8.45 M	0.20 M
		97.66%	68.19%	22.60 M	69.93%		18.59%	0.08 M
Calypso L18 3	39.18 M	38.29	53.26 M	26.84 M	37.35 M	43.57 M	9.69 M	0.26 M
		97.73%	69.55%	26.42 M	70.12%		18.20%	0.10 M

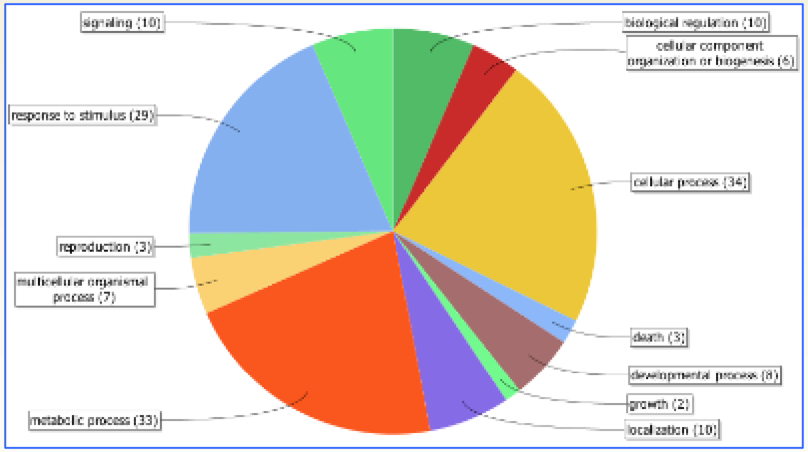
Differential gene and transcript expression analysis



RNA-Seq global data analysis and evaluation of differential gene expression

	LAA		GDL		Calypso				
						Differential expressed			
	Total	Diff.	Total	Diff.	Total	Global	Control - L5	Control - L18	L5 - L18
genes	33.404	211	33.458	617	33.874	179	67	84	28
isoforms	59.408	123	59.589	391	66.694	13	4	8	1
TSS	44.048	117	44.236	346	46.401	9	2	5	2
CDS	32.830	82	32.831	183	32.830	14	5	7	2
promoters	33.404	6	33.458	24	101.622	24	24	24	24
splicing	44.048	31	44.236	35	139.203	258	258	258	258
relCDS	31.344	1	31.373	5	93.300	4	4	4	4

GO Terms Biological process level 2

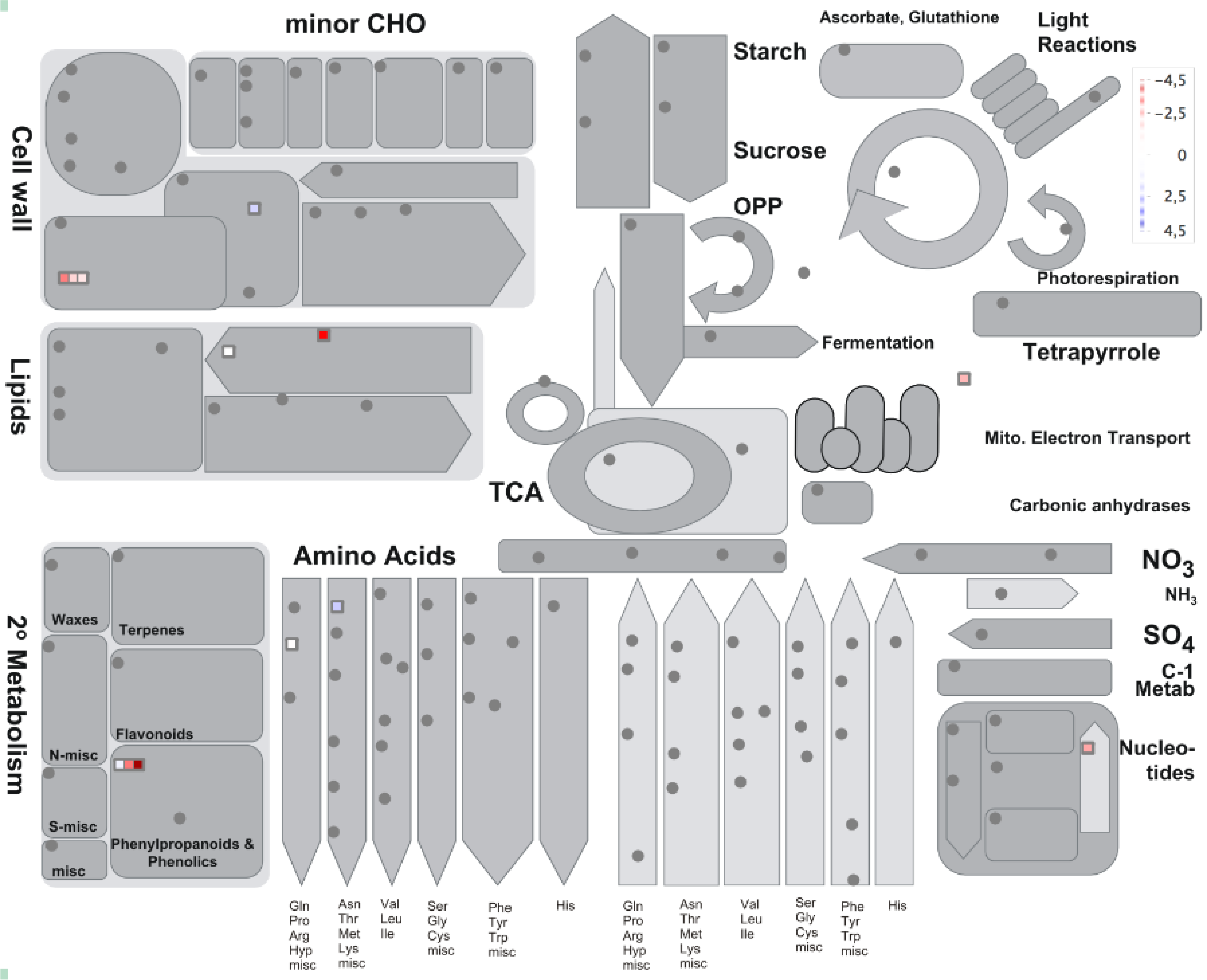


Down-regulated in L5 and L18, Significantly in both	49
Down-regulated in L5 and L18, only significant in L5	9
Down-regulated in L5 and L18, only significant in L18	25
Down-regulated in L5 and L18, only significant in L18, and L5 significatly differ from L18	5
Up-regulated in L5 and L18, Significantly in both	1
Up-regulated in L5 and L18, only significant in L5	7

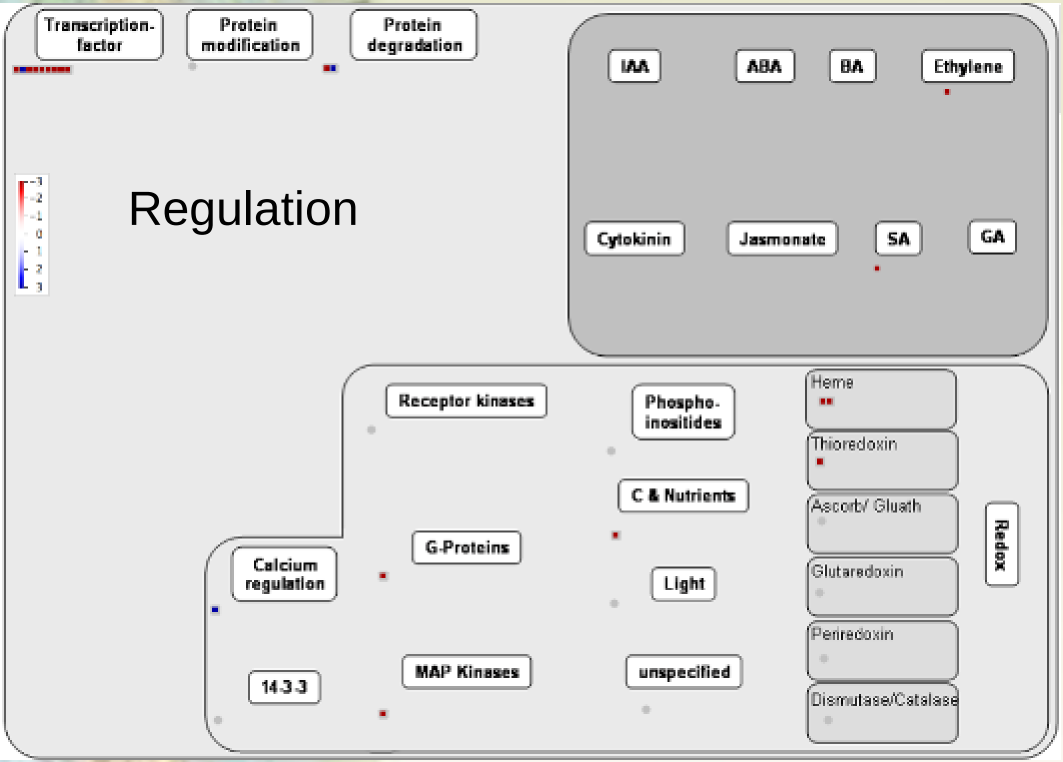
F. vesca Genemodel	Function	Control	L5	L18	change C-L5	Change C-L18	Mean C-Trans	Change L5-L18
gene12568-v1.0-hybrid	Phosphate-induced protein	16,2491	0,0518173	0,0262932	**-8,29271**	**-9,27145**	-8,78208	-0,978745
LOC101296541	uncharacterized	22,5352	0,110548	0,0327037	**-7,67135**	**-9,42851**	-8,54993	-1,75715
gene30244-v1.0-hybrid	2-aminoethanethiol dioxygenase like	39,8207	0,228369	0,101996	**-7,44601**	**-8,60887**	-8,02744	-1,16286
gene11662-v1.0-hybrid	UPF0481 protein At3g47200 like	240,074	1,57181	0,798593	**-7,25491**	**-8,23181**	-7,74336	-0,976899
gene29457-v1.0-hybrid	hypothetical protein PRUPE_ppa013785mg [Prunus persica]	202,783	0,675802	2,27703	**-8,22912**	**-6,47664**	-7,35288	1,75248
gene31571-v1.0-hybrid	Universal stress protein A-like protein	1734,81	12,3554	13,431	**-7,13349**	**-7,01307**	-7,07328	0,120428
gene25051-v1.0-hybrid	DUF3774; Wound-induced protein	651,548	4,103	6,2262	**-7,31105**	**-6,70938**	-7,010215	0,601674
gene19627-v1.0-hybrid	--NA--	67,0291	0,62976	0,742364	**-6,73384**	**-6,49652**	-6,61518	0,237325
gene30099-v1.0-hybrid	Myb_DNA-bind_4; Myb/SANT-like DNA-binding domain	216,058	1,70121	2,88429	**-6,98872**	**-6,22706**	-6,60789	0,761658
gene10187-v1.0-hybrid	DUF3774; Wound-induced protein	1027,95	8,62124	13,3662	**-6,89766**	**-6,26504**	-6,58135	0,632624
gene34341-v1.0-hybrid	MULE transposase domain	47,846	0,258664	1,09704	**-7,53118**	**-5,4467**	-6,48894	2,08447
gene26386-v1.0-hybrid	Kelch repeat-containing protein At3g27220 like	12,339	0,0886131	0,267725	**-7,12149**	**-5,52633**	-6,32391	1,59516
gene25053-v1.0-hybrid	--NA--	571,462	5,74765	9,50471	**-6,63554**	**-5,90987**	-6,272705	0,725669
gene04237-v1.0-hybrid	PI-PLC X domain-containing protein At5g67130, Precursor (similar to)	26,7978	0,389559	0,387558	**-6,10413**	**-6,11156**	-6,107845	-0,00743142
gene03436-v1.0-hybrid	uncharacterized LOC101302630	224,021	3,54184	3,46344	**-5,98299**	**-6,01529**	-5,99914	-0,0322948
gene24970-v1.0-hybrid	cinnamyl alcohol dehydrogenase-like protein	6,10426	0,127387	0,0762022	**-5,58252**	**-6,32384**	-5,95318	-0,741315
gene06111-v1.0-hybrid	uncharacterized LOC101295705	1011,61	15,7309	26,4924	**-6,00691**	**-5,25494**	-5,630925	0,751977
gene30986-v1.0-hybrid	Early nodulin-93 (N-93)	37,4341	1,13337	2,35084	**-5,04566**	**-3,99311**	-4,519385	1,05256
gene30069-v1.0-hybrid	Alcohol dehydrogenase	83,5689	3,09692	4,4626	**-4,75406**	**-4,22701**	-4,490535	0,527052
gene11086-v1.0-hybrid	ethylene-responsive transcription factor ERF071-like isoform 2	119,939	5,88124	4,93357	**-4,35004**	**-4,60353**	-4,476785	-0,253489
gene30740-v1.0-hybrid	B-box type zinc finger-containing protein	17,9052	0,808334	0,869763	**-4,46928**	**-4,36361**	-4,416445	0,10567
gene28088-v1.0-hybrid	--NA--	10,4113	0,255947	1,02692	**-5,34617**	**-3,34177**	-4,34397	2,0044
gene31963-v1.0-hybrid	hypothetical protein PRUPE_ppa018802mg, partial [Prunus persica]	397,95	24,1336	16,9444	**-4,04347**	**-4,5537**	-4,298585	-0,51023
gene18914-v1.0-hybrid	uncharacterized LOC101304561	123,842	10,1858	4,11561	**-3,60387**	**-4,91125**	-4,25756	-1,30738
gene08771-v1.0-hybrid	group 2 truncated hemoglobin YjbI-like	482,608	35,5565	21,961	**-3,76267**	**-4,45783**	-4,11025	-0,695166
gene10300-v1.0-hybrid	LOB domain-containing protein 41 like	135,768	9,7773	7,62028	**-3,79556**	**-4,15515**	-3,975355	-0,359592
gene19672-v1.0-hybrid	plastid division protein pdv2-like	157,675	10,9724	10,878	**-3,84501**	**-3,85747**	-3,85124	-0,0124595
gene19959-v1.0-hybrid	haloacid dehalogenase-like hydrolase domain-containing protein 3-like	246,09	17,1028	20,5349	**-3,84688**	**-3,58304**	-3,71496	0,263842
gene10643-v1.0-hybrid	DUF581; Protein of unknown function (DUF581)	272,999	20,2728	23,1962	**-3,75128**	**-3,55694**	-3,65411	0,194341
gene32701-v1.0-hybrid	acyl-[acyl-carrier-protein] desaturase 6, chloroplastic-like	12,0843	0,863998	1,14665	**-3,80597**	**-3,39764**	-3,601805	0,408329
gene30068-v1.0-hybrid	alcohol dehydrogenase 1-like	2532,51	243,321	226,755	**-3,37964**	**-3,48136**	-3,4305	-0,101722
gene08578-v1.0-hybrid	prolyl 4-hydroxylase subunit alpha-2-like	205,534	25,5746	21,0123	**-3,0066**	**-3,29007**	-3,148335	-0,283476
gene31964-v1.0-hybrid	Guanine nucleotide-binding protein alpha-2 subunit (GP-alpha-2)	39,674	4,97837	4,12445	**-2,99445**	**-3,26592**	-3,130185	-0,271472
gene01257-v1.0-hybrid	2-aminoethanethiol dioxygenase like	29,0116	3,7127	3,87596	**-2,96609**	**-2,90401**	-2,93505	0,0620841
gene11421-v1.0-hybrid	1-aminocyclopropane-1-carboxylate oxidase (ACC oxidase)	372,618	49,1362	51,2809	**-2,92284**	**-2,86121**	-2,892025	0,0616338
gene12910-v1.0-hybrid	DUF581; Protein of unknown function (DUF581)	113,456	12,5798	25,0435	**-3,17295**	**-2,17962**	-2,676285	0,993324
gene09243-v1.0-hybrid	cinnamyl alcohol dehydrogenase-like protein	272,14	67,1034	28,3249	**-2,01989**	**-3,26421**	-2,64205	-1,24432
gene24011-v1.0-hybrid	uncharacterized protein LOC101304806	325,289	48,4058	65,3015	**-2,74847**	**-2,31653**	-2,5325	0,431936
gene26038-v1.0-hybrid	Thioredoxin-like 1	487,352	76,2987	95,4731	**-2,67523**	**-2,3518**	-2,513515	0,323436
gene10366-v1.0-hybrid	ankyrin repeat-containing protein at5g02620- transcript variant 1	24,3282	4,53213	4,64742	**-2,42436**	**-2,38812**	-2,40624	0,0362404
gene22198-v1.0-hybrid	Hypoxia induced protein conserved region – Membrane	1047,18	218,32	198,219	**-2,26199**	**-2,40134**	-2,331665	-0,139351
gene01903-v1.0-hybrid	uncharacterized LOC101293650	130,662	31,0925	25,8304	**-2,0712**	**-2,3387**	-2,20495	-0,267496
gene14263-v1.0-hybrid	response to karrikin	273,828	63,2209	62,1071	**-2,11479**	**-2,14044**	-2,127615	-0,0256442
gene05802-v1.0-hybrid	phytosulfokines 3-like	481,036	106,771	117,198	**-2,17162**	**-2,0372**	-2,10441	0,134423

F. vesca Genemodel	Function	Control	L5	L18	change C-L5	Change C-L18	Mean C-Trans	Change L5-L18
gene12568-v1.0-hybrid	Phosphate-induced protein	16,2491	0,0518173	0,0262932	**-8,29271**	**-9,27145**	-8,78208	-0,978745
LOC101296541	uncharacterized	22,5352	0,110548	0,0327037	**-7,67135**	**-9,42851**	-8,54993	-1,75715
gene30244-v1.0-hybrid	2-aminoethanethiol dioxygenase like	39,8207	0,228369	0,101996	**-7,44601**	**-8,60887**	-8,02744	-1,16286
gene11662-v1.0-hybrid	UPF0481 protein At3g47200 like	240,074	1,57181	0,798593	**-7,25491**	**-8,23181**	-7,74336	-0,976899
gene29457-v1.0-hybrid	hypothetical protein PRUPE_ppa013785mg [Prunus persica]	202,783	0,675802	2,27703	**-8,22912**	**-6,47664**	-7,35288	1,75248
gene31571-v1.0-hybrid	Universal stress protein A-like protein	1734,81	12,3554	13,431	**-7,13349**	**-7,01307**	-7,07328	0,120428
gene25051-v1.0-hybrid	DUF3774; Wound-induced protein	651,548	4,103	6,2262	**-7,31105**	**-6,70938**	-7,010215	0,601674
gene19627-v1.0-hybrid	--NA--	67,0291	0,62976	0,742364	**-6,73384**	**-6,49652**	-6,61518	0,237325
gene30099-v1.0-hybrid	Myb_DNA-bind_4; Myb/SANT-like DNA-binding domain	216,058	1,70121	2,88429	**-6,98872**	**-6,22706**	-6,60789	0,761658
gene10187-v1.0-hybrid	DUF3774; Wound-induced protein	1027,95	8,62124	13,3662	**-6,89766**	**-6,26504**	-6,58135	0,632624
gene34341-v1.0-hybrid	MULE transposase domain	47,846	0,258664	1,09704	**-7,53118**	**-5,4467**	-6,48894	2,08447
gene26386-v1.0-hybrid	Kelch repeat-containing protein At3g27220 like	12,339	0,0886131	0,267725	**-7,12149**	**-5,52633**	-6,32391	1,59516
gene25053-v1.0-hybrid	--NA--	571,462	5,74765	9,50471	**-6,63554**	**-5,90987**	-6,272705	0,725669
gene04237-v1.0-hybrid	PI-PLC X domain-containing protein At5g67130, Precursor (similar to)	26,7978	0,389559	0,387558	**-6,10413**	**-6,11156**	-6,107845	-0,00743142
gene03436-v1.0-hybrid	uncharacterized LOC101302630	224,021	3,54184	3,46344	**-5,98299**	**-6,01529**	-5,99914	-0,0322948
gene24970-v1.0-hybrid	cinnamyl alcohol dehydrogenase-like protein	6,10426	0,127387	0,0762022	**-5,58252**	**-6,32384**	-5,95318	-0,741315
gene06111-v1.0-hybrid	uncharacterized LOC101295705	1011,61	15,7309	26,4924	**-6,00691**	**-5,25494**	-5,630925	0,751977
gene30986-v1.0-hybrid	Early nodulin-93 (N-93)	37,4341	1,13337	2,35084	**-5,04566**	**-3,99311**	-4,519385	1,05256
gene30069-v1.0-hybrid	Alcohol dehydrogenase	83,5689	3,09692	4,4626	**-4,75406**	**-4,22701**	-4,490535	0,527052
gene11086-v1.0-hybrid	ethylene-responsive transcription factor ERF071-like isoform 2	119,939	5,88124	4,93357	**-4,35004**	**-4,60353**	-4,476785	-0,253489
gene30740-v1.0-hybrid	B-box type zinc finger-containing protein	17,9052	0,808334	0,869763	**-4,46928**	**-4,36361**	-4,416445	0,10567
gene28088-v1.0-hybrid	--NA--	10,4113	0,255947	1,02692	**-5,34617**	**-3,34177**	-4,34397	2,0044
gene31963-v1.0-hybrid	hypothetical protein PRUPE_ppa018802mg, partial [Prunus persica]	397,95	24,1336	16,9444	**-4,04347**	**-4,5537**	-4,298585	-0,51023
gene18914-v1.0-hybrid	uncharacterized LOC101304561	123,842	10,1858	4,11561	**-3,60387**	**-4,91125**	-4,25756	-1,30738
gene08771-v1.0-hybrid	group 2 truncated hemoglobin YjbI-like	482,608	35,5565	21,961	**-3,76267**	**-4,45783**	-4,11025	-0,695166
gene10300-v1.0-hybrid	LOB domain-containing protein 41 like	135,768	9,7773	7,62028	**-3,79556**	**-4,15515**	-3,975355	-0,359592
gene19672-v1.0-hybrid	plastid division protein pdv2-like	157,675	10,9724	10,878	**-3,84501**	**-3,85747**	-3,85124	-0,0124595
gene19959-v1.0-hybrid	haloacid dehalogenase-like hydrolase domain-containing protein 3-like	246,09	17,1028	20,5349	**-3,84688**	**-3,58304**	-3,71496	0,263842
gene10643-v1.0-hybrid	DUF581; Protein of unknown function (DUF581)	272,999	20,2728	23,1962	**-3,75128**	**-3,55694**	-3,65411	0,194341
gene32701-v1.0-hybrid	acyl-[acyl-carrier-protein] desaturase 6, chloroplastic-like	12,0843	0,863998	1,14665	**-3,80597**	**-3,39764**	-3,601805	0,408329
gene30068-v1.0-hybrid	alcohol dehydrogenase 1-like	2532,51	243,321	226,755	**-3,37964**	**-3,48136**	-3,4305	-0,101722
gene08578-v1.0-hybrid	prolyl 4-hydroxylase subunit alpha-2-like	205,534	25,5746	21,0123	**-3,0066**	**-3,29007**	-3,148335	-0,283476
gene31964-v1.0-hybrid	Guanine nucleotide-binding protein alpha-2 subunit (GP-alpha-2)	39,674	4,97837	4,12445	**-2,99445**	**-3,26592**	-3,130185	-0,271472
gene01257-v1.0-hybrid	2-aminoethanethiol dioxygenase like	29,0116	3,7127	3,87596	**-2,96609**	**-2,90401**	-2,93505	0,0620841
gene11421-v1.0-hybrid	1-aminocyclopropane-1-carboxylate oxidase (ACC oxidase)	372,618	49,1362	51,2809	**-2,92284**	**-2,86121**	-2,892025	0,0616338
gene12910-v1.0-hybrid	DUF581; Protein of unknown function (DUF581)	113,456	12,5798	25,0435	**-3,17295**	**-2,17962**	-2,676285	0,993324
gene09243-v1.0-hybrid	cinnamyl alcohol dehydrogenase-like protein	272,14	67,1034	28,3249	**-2,01989**	**-3,26421**	-2,64205	-1,24432
gene24011-v1.0-hybrid	uncharacterized protein LOC101304806	325,289	48,4058	65,3015	**-2,74847**	**-2,31653**	-2,5325	0,431936
gene26038-v1.0-hybrid	Thioredoxin-like 1	487,352	76,2987	95,4731	**-2,67523**	**-2,3518**	-2,513515	0,323436
gene10366-v1.0-hybrid	ankyrin repeat-containing protein at5g02620- transcript variant 1	24,3282	4,53213	4,64742	**-2,42436**	**-2,38812**	-2,40624	0,0362404
gene22198-v1.0-hybrid	Hypoxia induced protein conserved region – Membrane	1047,18	218,32	198,219	**-2,26199**	**-2,40134**	-2,331665	-0,139351
gene01903-v1.0-hybrid	uncharacterized LOC101293650	130,662	31,0925	25,8304	**-2,0712**	**-2,3387**	-2,20495	-0,267496
gene14263-v1.0-hybrid	response to karrikin	273,828	63,2209	62,1071	**-2,11479**	**-2,14044**	-2,127615	-0,0256442
gene05802-v1.0-hybrid	phytosulfokines 3-like	481,036	106,771	117,198	**-2,17162**	**-2,0372**	-2,10441	0,134423

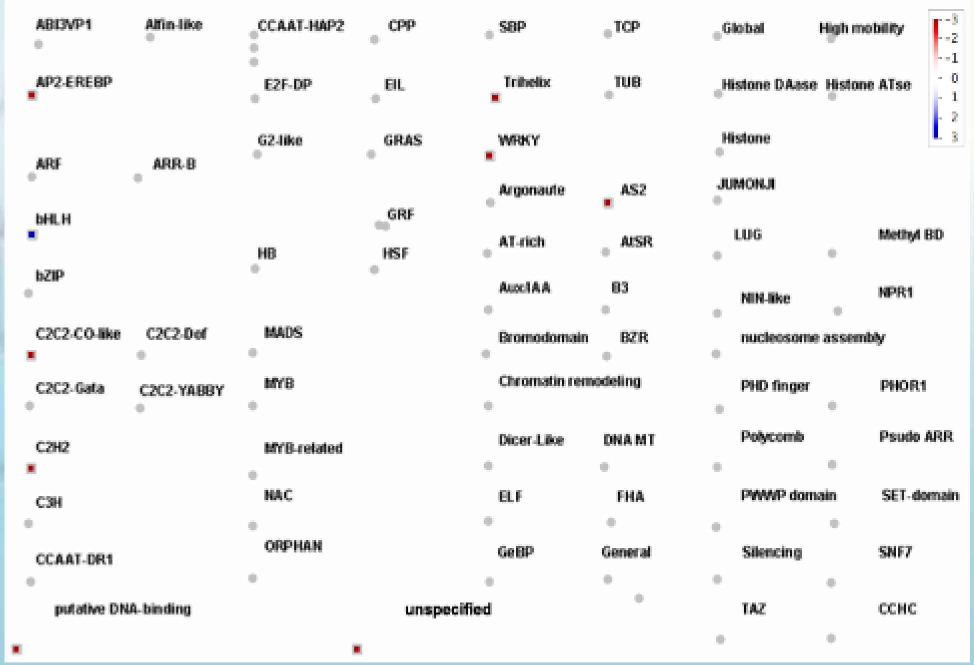
Metabolism overview



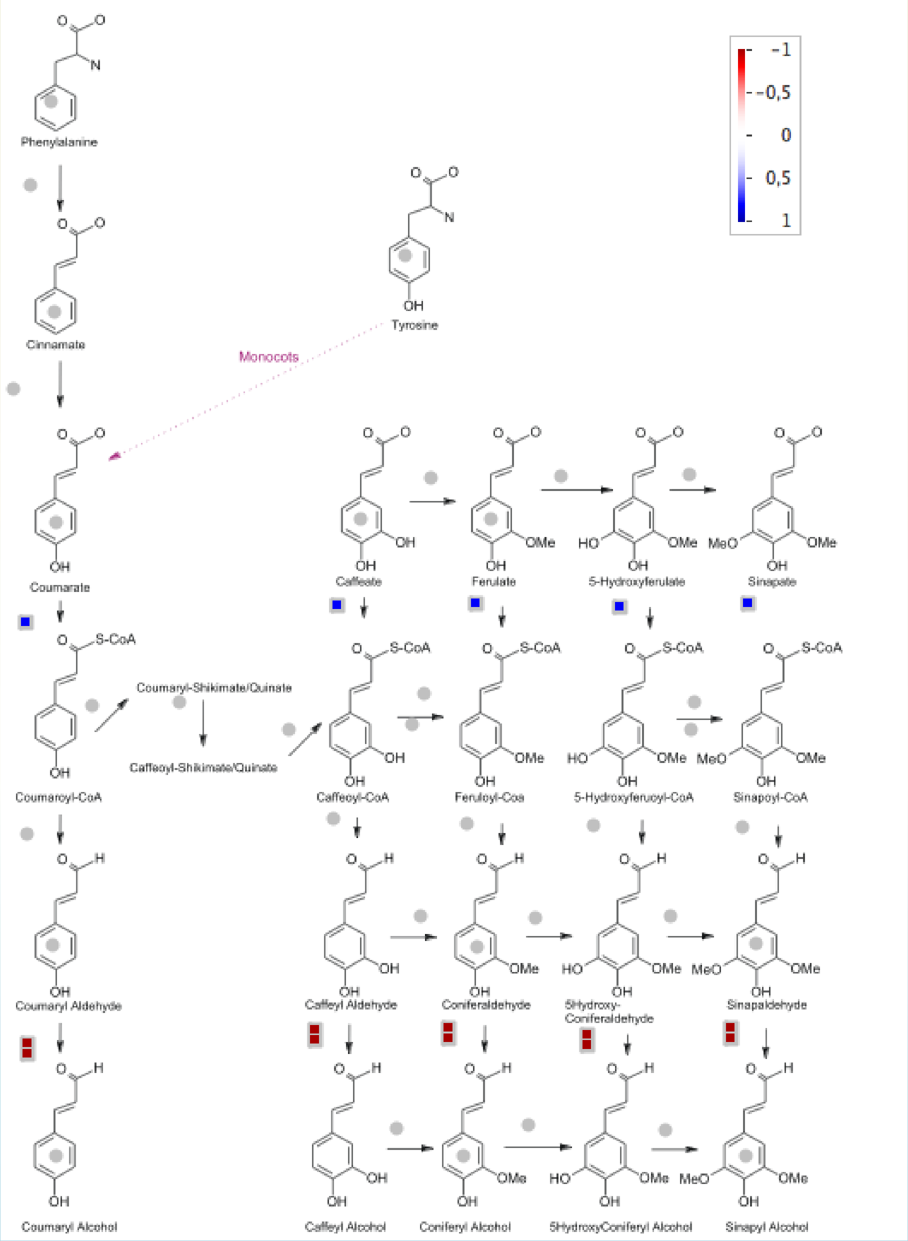
Regulation



Transcription factors



Phenylpropanoids Pathway



D3.3

Evidence for the human health benefits of fresh fruit in validated human digestion, bioavailability and disease model systems (P1, P3, P4)

Results of P1 GROUP

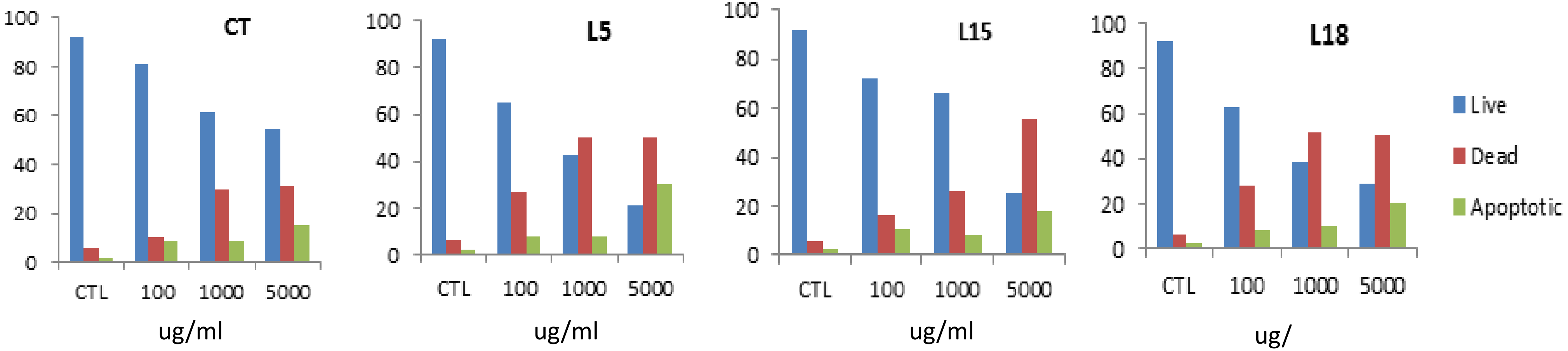
☐ **Study I:** Cellular functionality and redox balance on HepG2 cells treated with 3 different GMO selections.

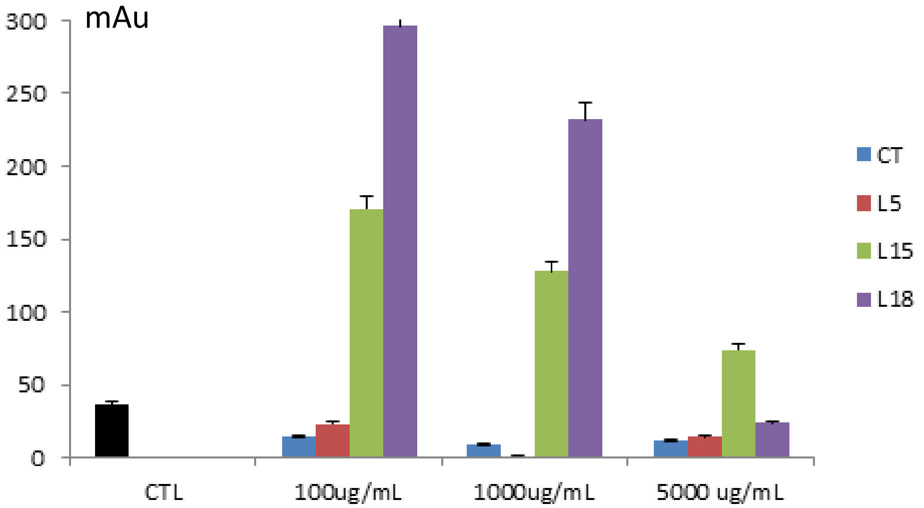
☐ **Study II:** Cellular functionality and redox balance on HDF cells stressed with AAPH and treated with 3 different GMO selections.

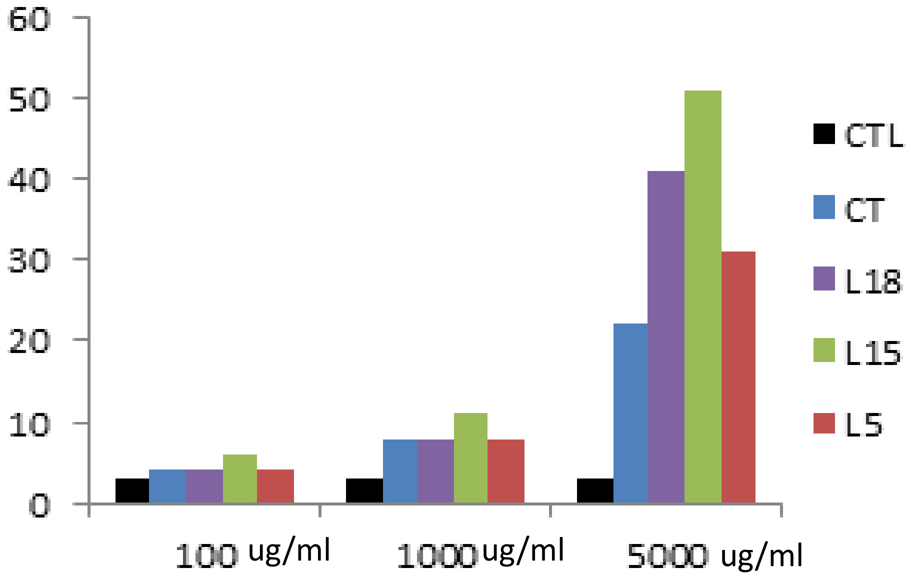
☐ **Study III:** Cytotoxic effect of berries IVD extracts in HepG2.

STUDY I

Cellular functionality and redox balance on HepG2 cells treated with 3 different GMO selections.

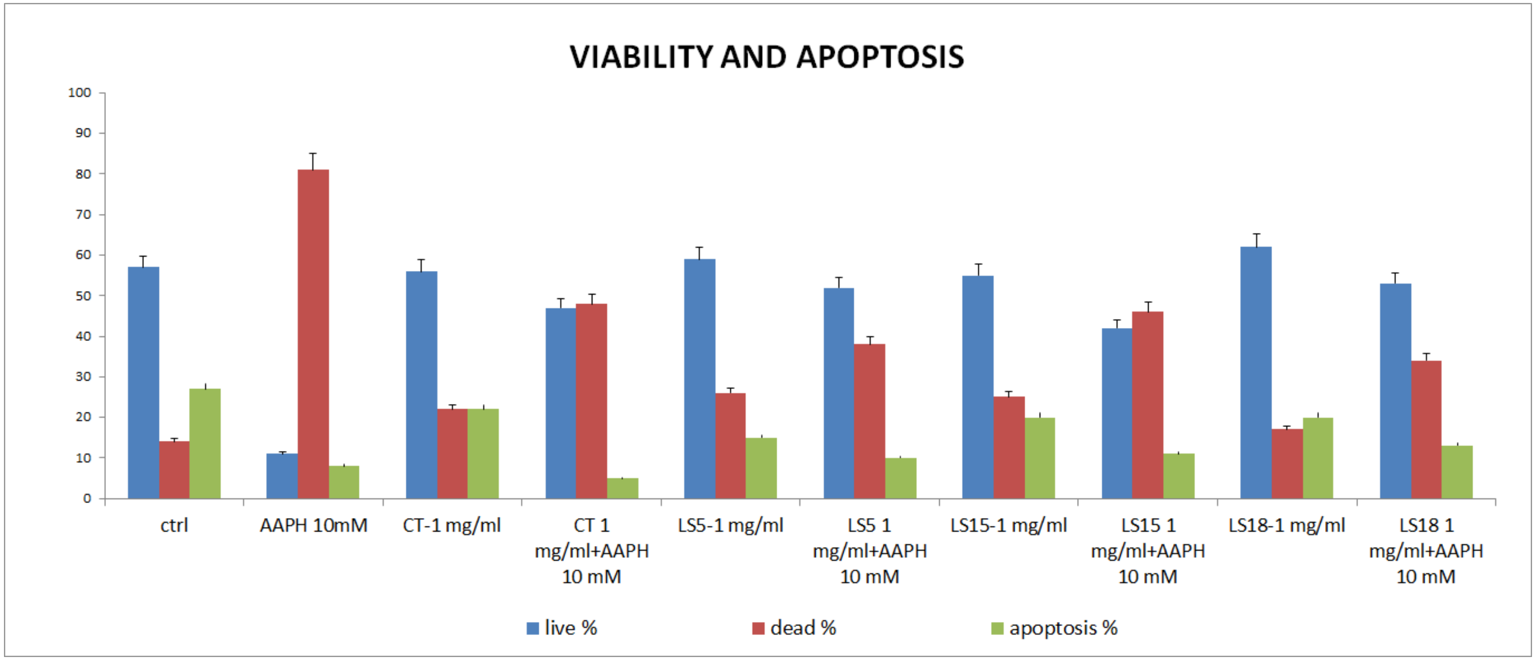


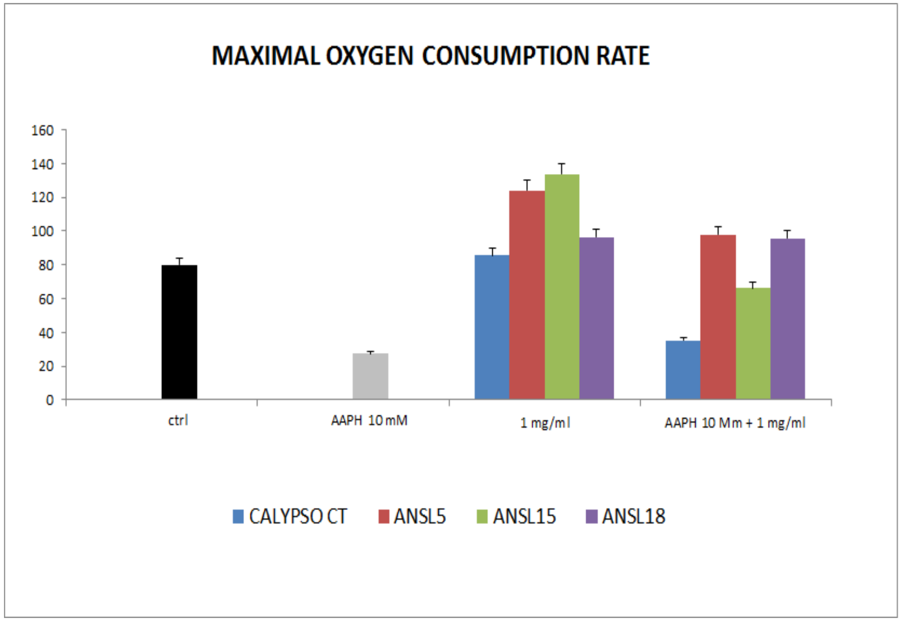


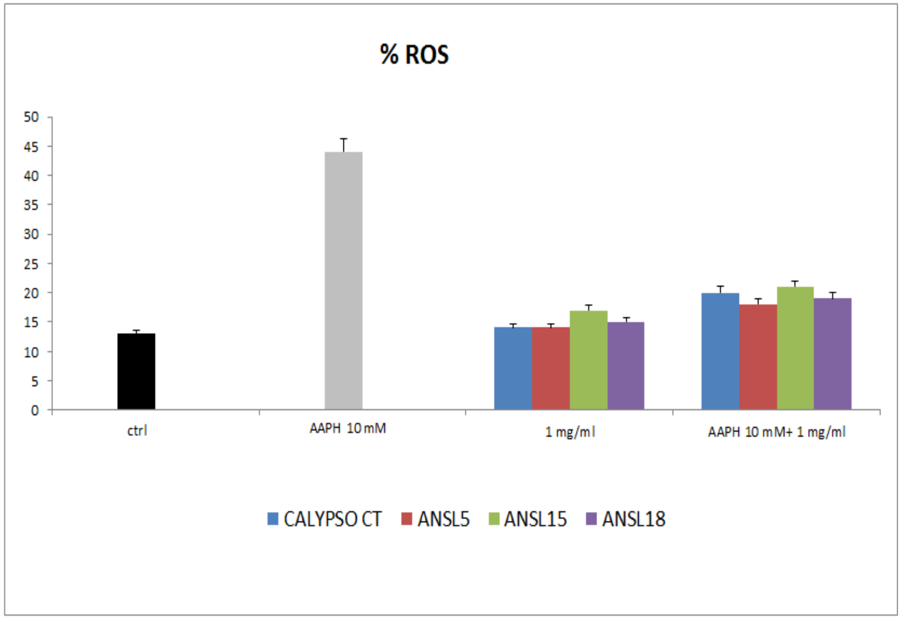


STUDY II

Cellular functionality and redox balance on HDF cells stressed with AAPH and treated with 3 different GMO selections

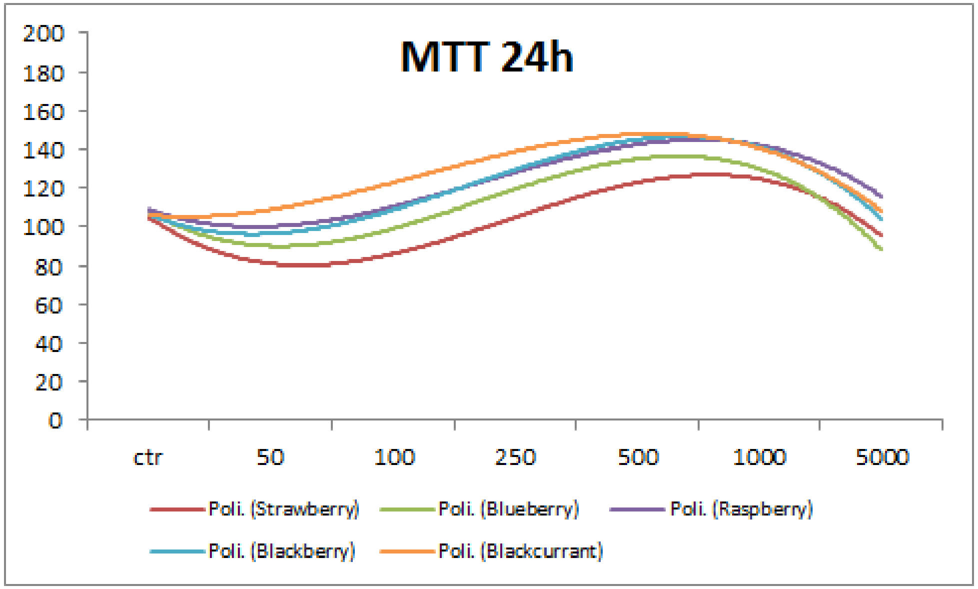

VIABILITY AND APOPTOSIS

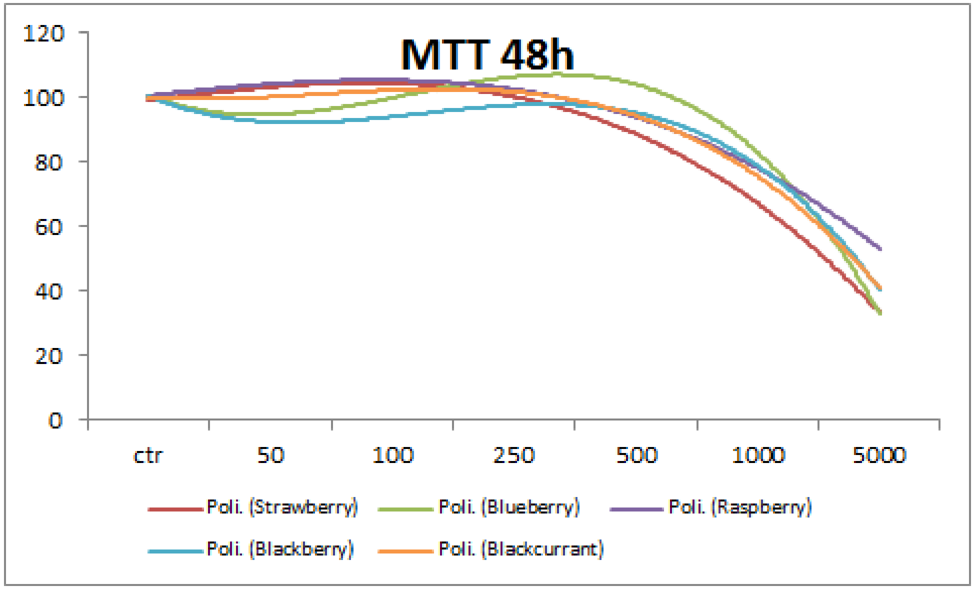

MAXIMAL OXYGEN CONSUMPTION RATE

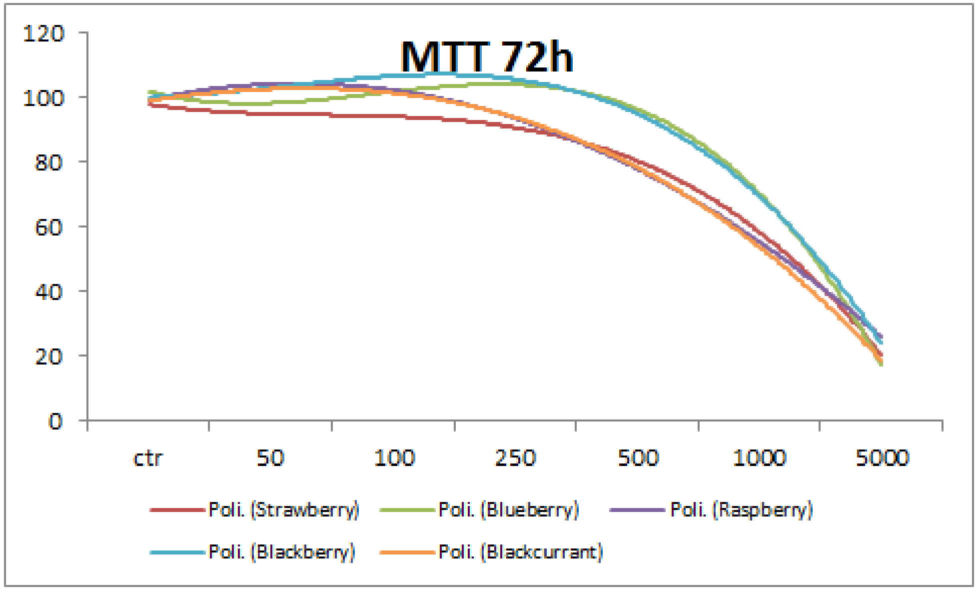

% ROS

STUDY III

Citotoxic effect of berries IVD extracts in HepG2

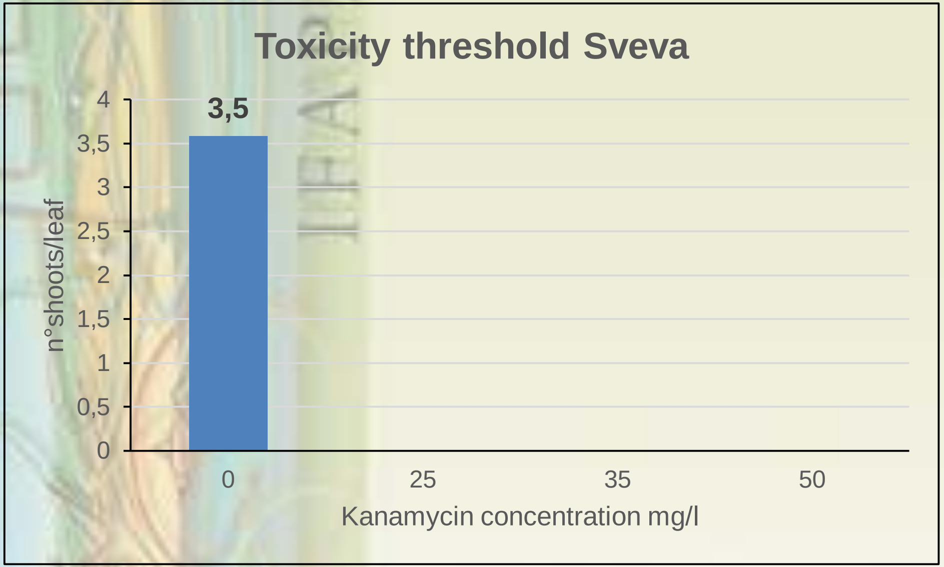




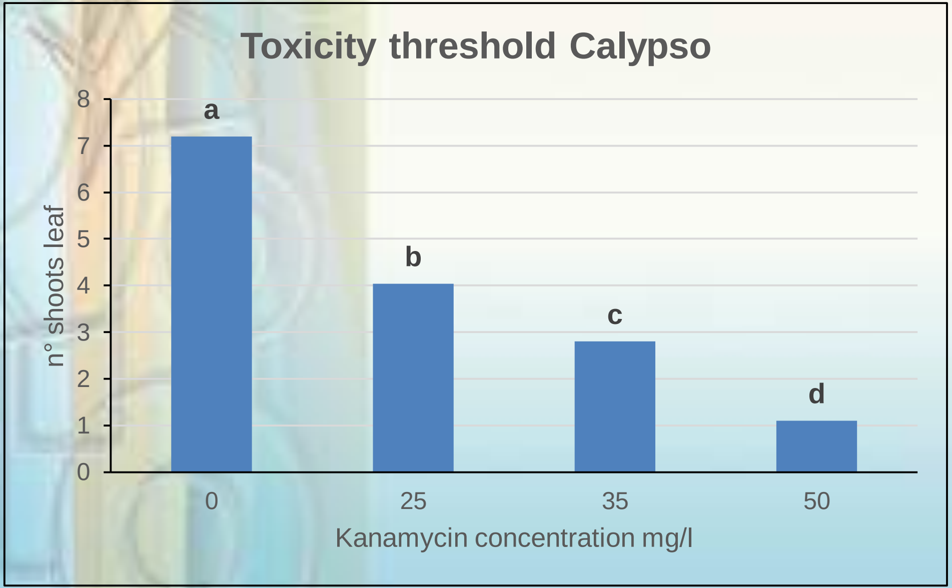


Sub-task 1.3.2 Validation of flowering-related genes

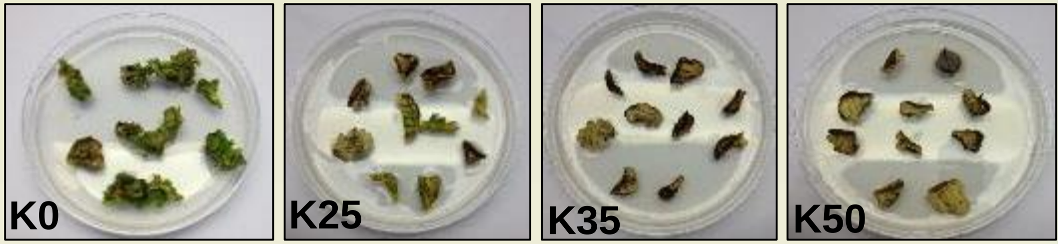
First step: *selective agent concentration, Kanamycin*

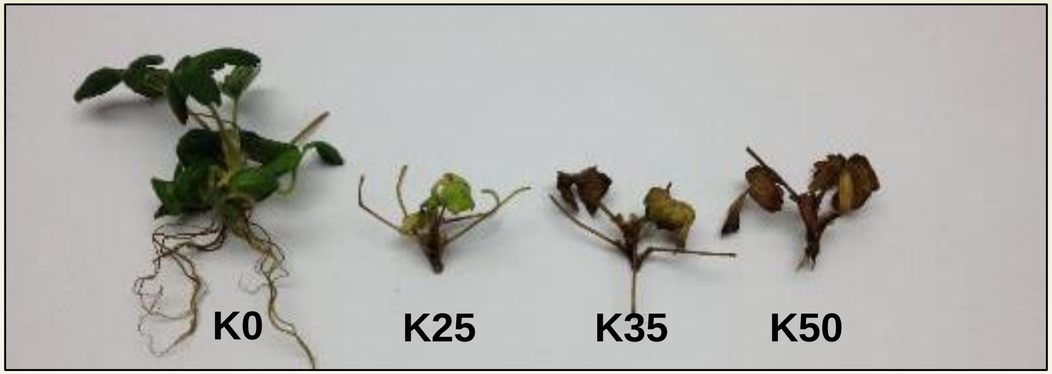


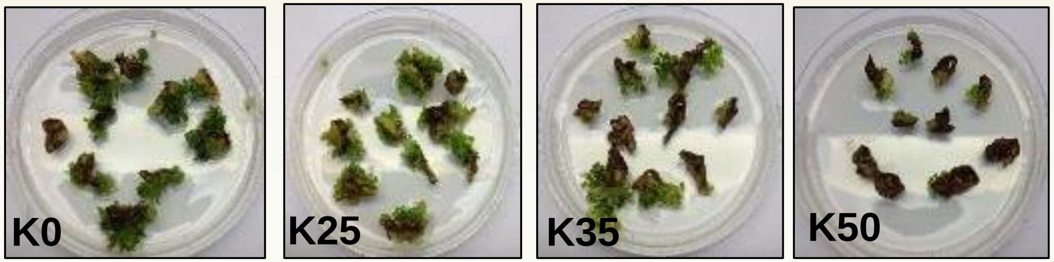
Sveva Kanamycin concentration used during transformation trials selection: 35 mg/l; during plant selection: 25 mg/l



Calypso Kanamycin concentration used during transformation trials selection: 50 mg/l; during plant selection: 75 mg/l



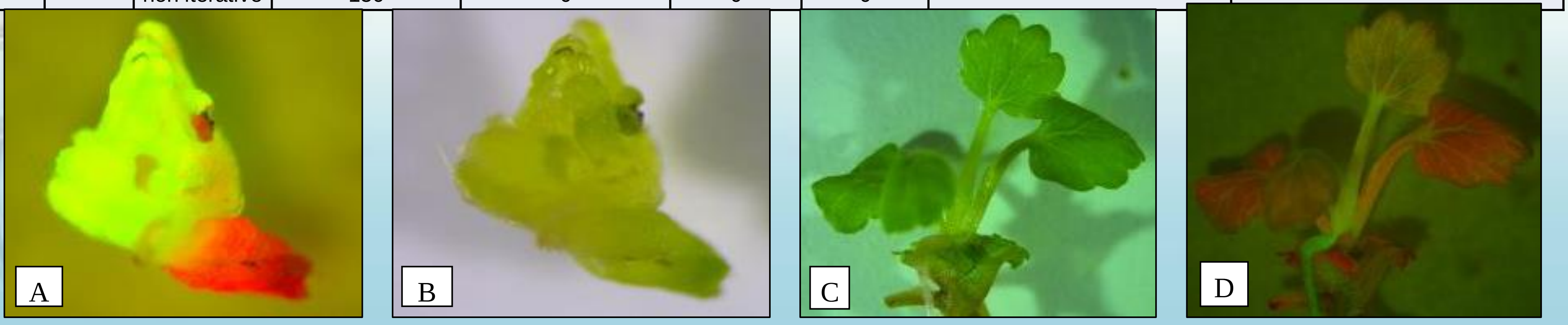




Third step : Transformation trials 2014. Protocol: (Cappelletti R. *et al.*, 2013 in press) Wang K. **Experimental procedure:** 100 leaves x 5 construct x 2 varieties = 3000 leaves. **Factors:** construct used. **Selection method:** Kanamycin selection parallel with GFP fluorescence monitoring **Variables:** n°regenerated lines; n°GFP$^+$ lines; n° GFP$^-$ lines; % regeneration; % putative transgenic lines;

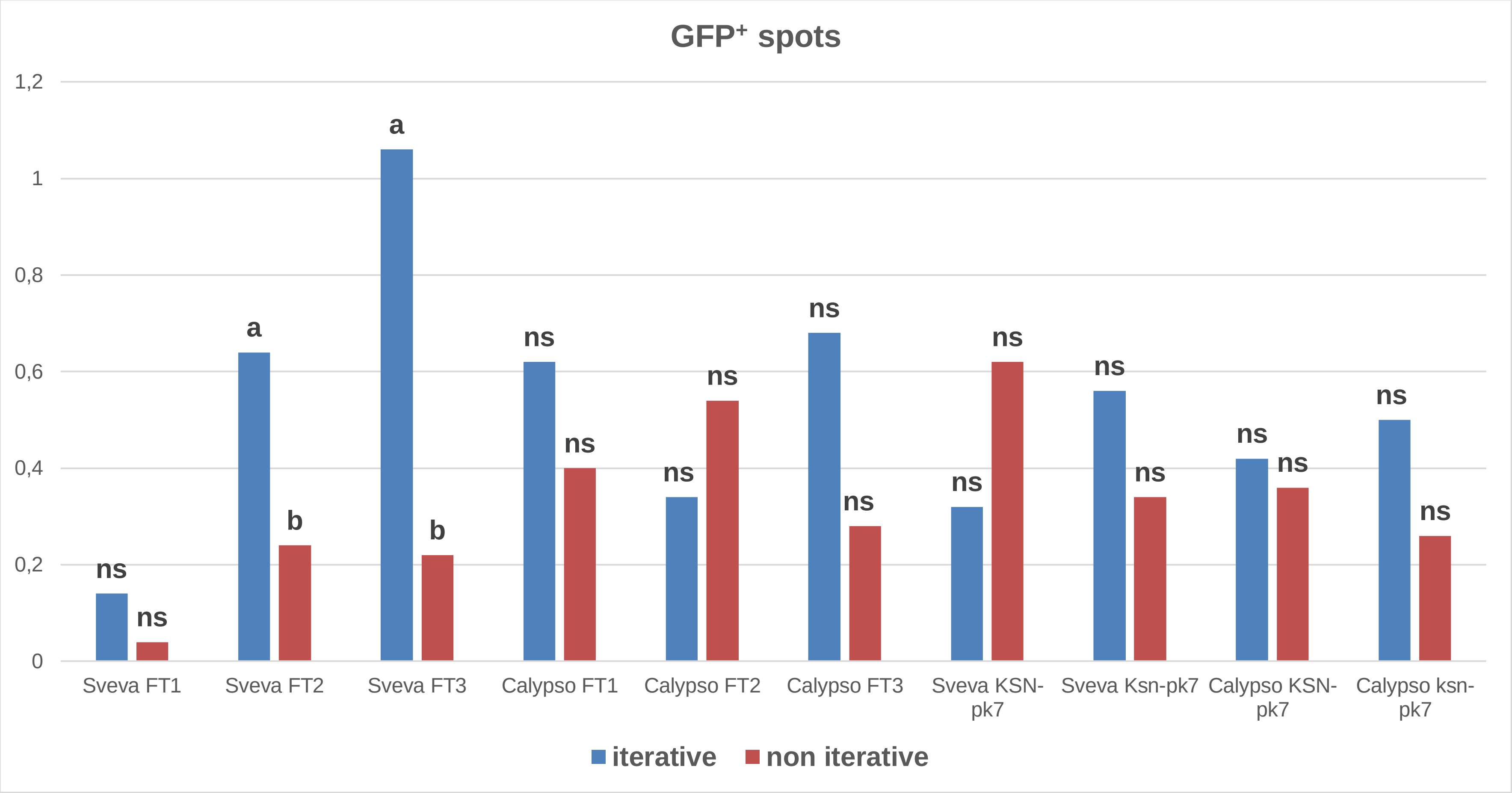
Sveva selection: iterative K25 → K50 mg/l, non iterative K35 mg/l. **Calypso selection:** iterative K25 → K50 mg/l, non iterative K50 mg/l

Construct	Variety	Selection	n° leaves infected	n°regenerated lines	n°GFP$^+$ lines	n°GFP$^-$ lines	% regeneration	% putative GFP$^+$ transgenic lines
FT1	Calypso	iterative	150	8	0	8	3,3	0,33
		non iterative	150	2	1	1		
	Sveva	iterative	150	3	3	0	1	1
		non iterative	150	0	0	0		
FT2	Calypso	iterative	150	10	0	10	4	0
		non iterative	150	3	0	3		
	Sveva	iterative	150	2	1	1	0,67	0,33
		non iterative	150	0	0	0		
FT3	Calypso	iterative	150	6	0	6	3	0
		non iterative	150	3	0	3		
	Sveva	iterative	150	1	0	1	0,33	0
		non iterative	150	0	0	0		
KSN	Calypso	iterative	150	5	1	4	2,67	0,33
		non iterative	150	3	0	3		
	Sveva	iterative	150	0	0	0	0	0
		non iterative	150	0	0	0		
ksn	Calypso	Iterative	150	8	1	7	3,67	0,33
		non iterative	150	3	0	3		
	Sveva	Iterative	150	0	0	0	0	0
		non iterative	150	0	0	0		



A-B→ GFP$^+$ meristematic dome of Calypso FT3 with chimeric area GFP$^-$. C-D → GFP$^+$ Sveva FT2 putative transgenic lines obtained under K35 mg/l kanamycin selection.

Fourth step: monitoring GFP spot of leaves under selection in two different selection method: iterative, progressively increase of kanamycin concentration K25 → K35 → K45 → K50; non iterative: concentration at 35 mg/l → Sveva, 50 mg/l → Calypso.



Fluorescence observation of putative transgenic line of Sveva FT2: A. Leaf on clear field B. Leaf GFP+ C. Sveva FT2 shoots clear field. D Sveva FT2 GFP+

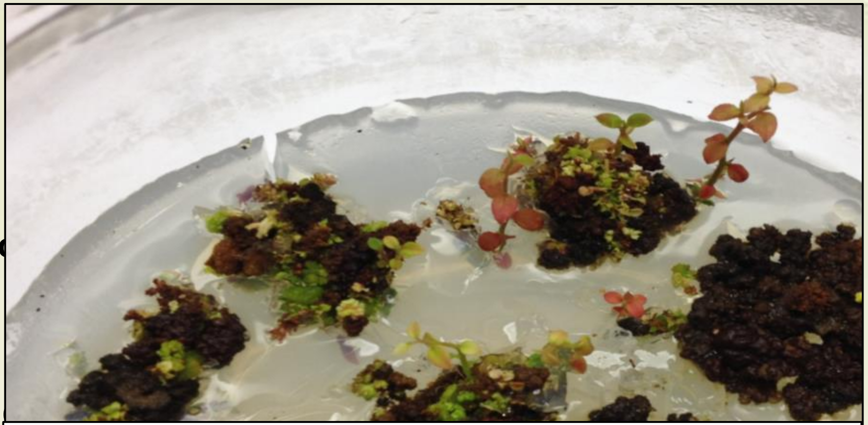
Sub-task 1.3.2 Blueberry micropropagation and regeneration

- in vitro establishment of Blueberry Duke, Earlyblue, Blueray and Bluecrop

- WPM + Zeatin 2 mg/l + 30 g/l Sucrose + 2-4 mg/l Zeatin

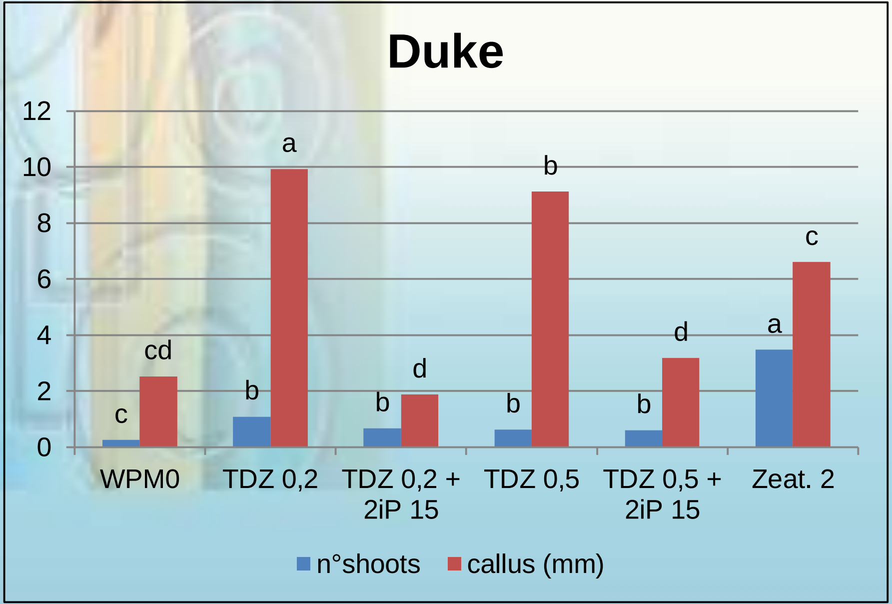
Proliferation and callus inducti

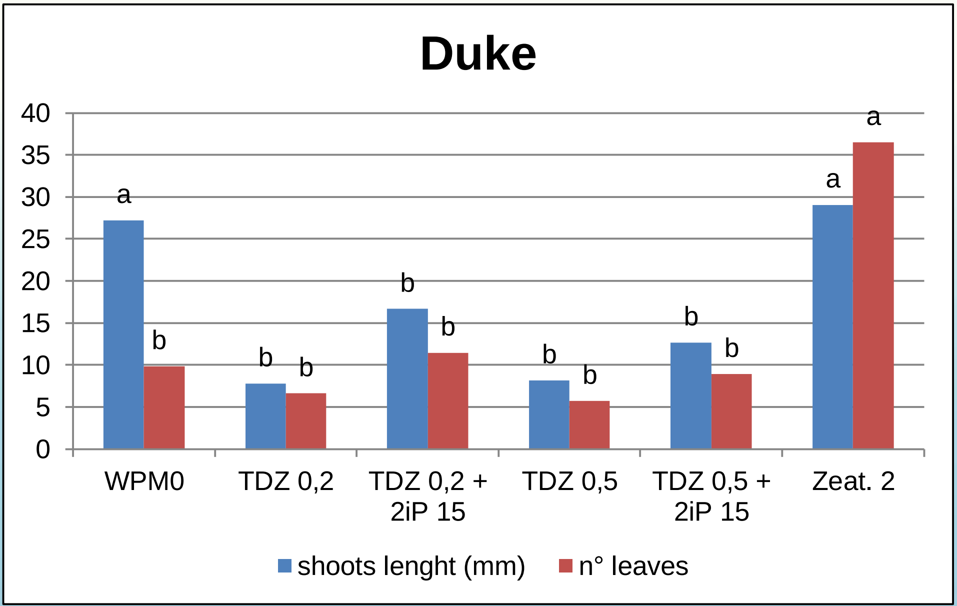
- WPM (control)
- WPM + Zeatin 2 mg/l
- WPM + TDZ 0,2 mg/l
- WPM + TDZ 0,2 mg/l + 2iP 15 m
- WPM + TDZ 0,5 mg/l
- WPM + TDZ 0,5 mg/l + 2iP 15 mg/l



Meristematic Bulk Blueberry Duke WPM + TDZ 0,5 mg/l

Leaf regeneration with 2 mg/l Zeatin





Thank you!